# 6/3/2016 -- Gen. Notes

Friday, June 3, 2016
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**General Plasmid Process**

Build plasmid --> cloning strain amplification --> purify plasmid --> uptake plasmid into expression strain

**Plasmid Purification Process**

1. Spin/pellet the cells and remove supernatant
2. Lyse and extract cells
    a. 250 uL of Buffer 1 (resuspends DNA) pipette into 1.5 mL tube
    b. 250 uL of Buffer 2 (lyses cells) then invert tube
    c. 350 uL of Buffer 3 (neutralizes cells) then invert tube
3. Spin and purify cells
    a. Spin for 10 minutes (DNE = supernatant)
    b. Load supernatant into column
        i. Bind DNA
        ii. Buffer A (inactivates enzymes from DNA degradation)
        iii. Wash Buffer
        iv. Elute with water or elution buffers

Cloning strains --> Top10 and DH5(alpha) (high transformation efficiencies)
Expression strains --> BL21 (need to overexpress proteins)
Expression and Genomic data --> MG1655

# 6/8/2016 -- Digests

Tuesday, June 14, 2016
3:39 PM

14A, 14C -- EcoRI, SpeI -- Cutsmart
-- EcoRI, XbaI, SAP -- Cutsmart
**Note:** Google NEB Double Digest Finder

***General Protocol***

250 ng DNA
2 uL Cutsmart
0.5 EcoRI
0.5 SpeI

--> water up to 20 uL

***14A***

7.12 uL DNA

8 uL CS
2 uL EcoRI
2 uL SpeI

60.88 uL water
***14C***

8.76 DNA
8 uL CS
2 uL EcoRI
2 uL SpeI

59.24 uL water
***1P***

7.00 uL DNA
8 uL CS
2 uL EcoRI
2 uL SpeI

61 uL water

Incubate 1 hour (37 degrees C)
Heat kill 20 min (80 degrees C)

8% gel, 100V, 40 min

# 6/9/2015 -- Enzyme Tests

Same protocol as 6/8
Needed to test enzyme due to abnormal results
Ran smaller digests
Tuesday, June 14, 2016
3:45 PM

| *Plasmid* | ***14A*** | ***14C*** | ***1P*** |
|---|---|---|---|
| *DNA* | 1.79 | 2.19 | 1.75 |
| *Cutsmart* | 2 | 2 | 2 |
| *SpeI/XbaI* | 0.5 SpeI | 0.5 SpeI | 0.5 XbaI |
| *EcoRI* | 0.5 | 0.5 | 0.5 |
| *Water* | 15.22 | 14.81 | 15.25 |

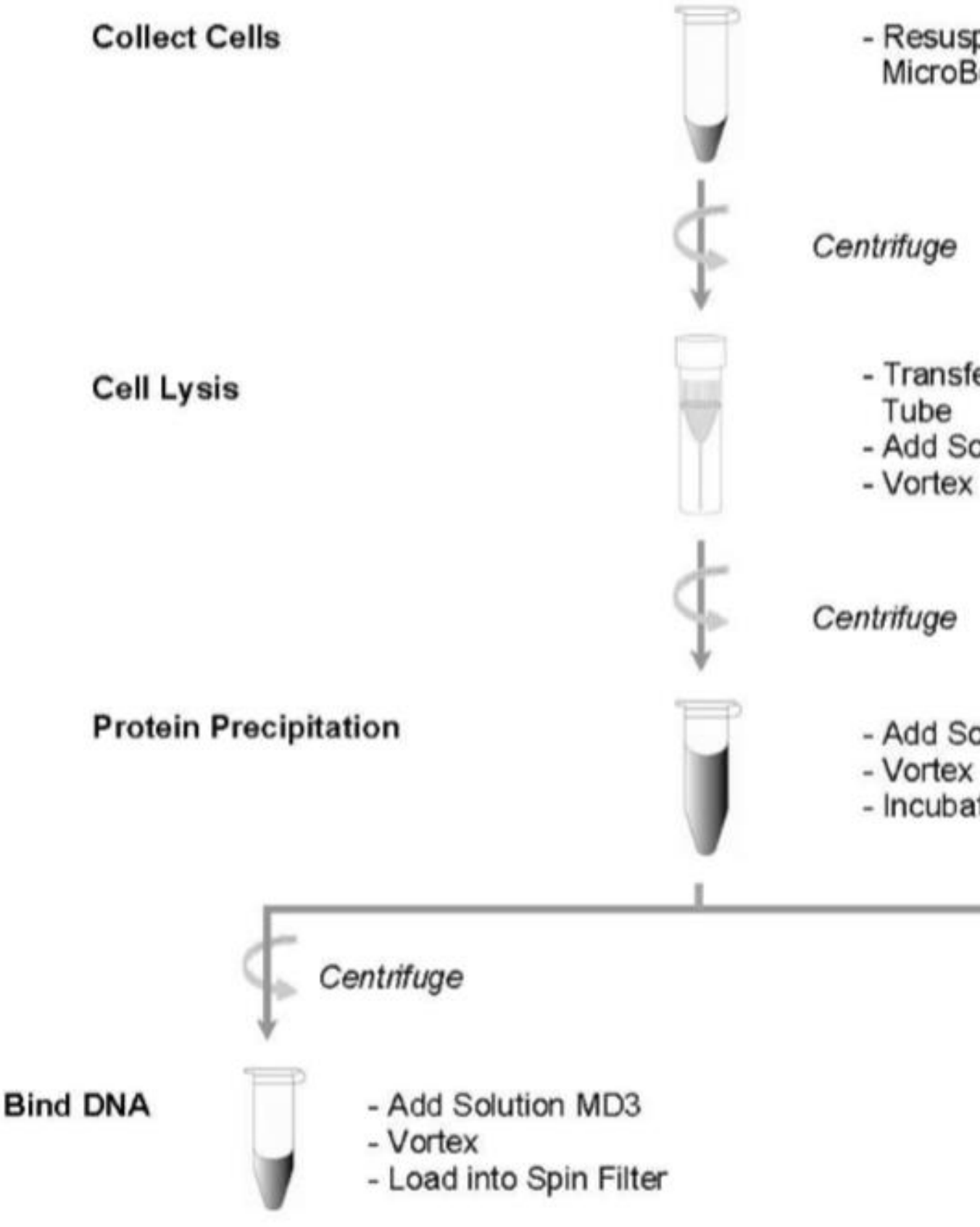
Incubate 37 degrees C for 1 hour
Heart shock 80 degrees C for 15 min

# 6/14/2016 -- DNA Isolation

Tuesday, June 14, 2016
3:53 PM

Ran protocol from UltraClean Microbial DNA Isolation Kit Instruction Manual
Used detailed protocol

# UltraClean® Microbial DNA Isola



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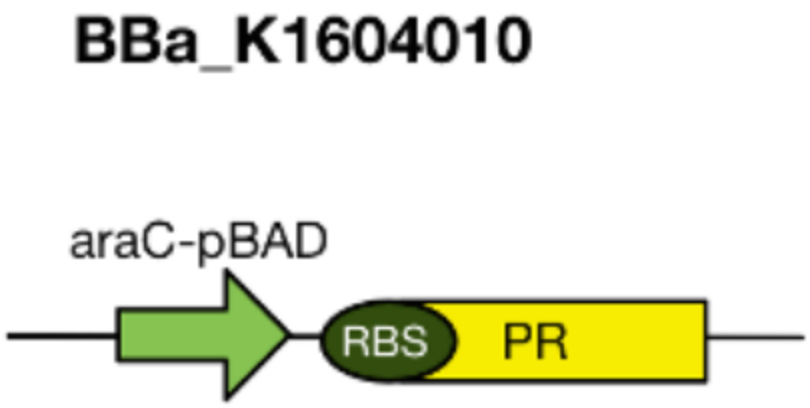


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# 7/11/2016 -- PCR (Primer+GFP)

Monday, July 11, 2016
1:56 PM

**General PCR Protocol**

https://www.neb.com/protocols/1/01/01/taq-dna-polymerase-with-standard-taq-buffer-m0273

- Setting the PCR thermocycler settings
  - Login as iGEM (password = igem)
  - Click on the iGEM folder, select a protocol (or make a new one), and adjust the settings
  - A temperature gradient can be created to cover a range of annealing temperatures

- Preparing PCR solution
  - Get a container and fill it with ice. PCR components should be mixed in test tubes on ice.
  - Mix the following concentrations according to the NEB PCR Kit
  - Ex:

| Component | 25 µL Rxn | 50 µL Rxn |
|---|---|---|
| 10 µM Forward Primer | 1.25 µL | 2.5 µL |
| 10 µM Reverse Primer | 1.25 µL | 2.5 µL |

| | | |
|---|---|---|
| Template DNA | | |
| Q5 High Fidelity 2X Master Mix | 12.5 µL | 25 µL |
| Nuclease Free Water | to 25 µL | to 50 µL |

Promoter PCR list for Viraat and I:

| | | |
|---|---|---|
| iGEM8 | For Prom 2531 | Add 308 uL for 100 uM |
| iGEM9 | Rev Prom 2531 | Add 279 uL for 100 uM |
| iGEM12 | For Prom 1224 | Add 305 uL for 100 uM |
| iGEM13 | Rev Prom 1224 | Add 288 uL for 100 uM |
| iGEM22 | For Prom 3517 | Add 276 uL for 100 uM |
| iGEM23 | Rev Prom 3517 | Add 288 uL for 100 uM |
| Template DNA | | 119.2 ng/uL Concentration |
| GFP Block | | 0.1 ng/uL |
| GFP_For | | Add 279 uL for 100 uM |
| GFP_Rev | | Add 292 uL for 100 uM |

Centrifuge the 100 uM primers and resuspend/dilute with DI water in 1:10 ratio to get 10 uM.

## Today's PCR Protocol

| Component | 25 uL rxn |
|---|---|
| GFP_For | 1.25 uL |
| GFP_Rev | 1.25 uL |
| 2D Diluted GFP_Block (0.1 ng/uL) | |
| Master Mix | |
| DI Water | |

| Component | 25 uL rxn |
|---|---|
| iGEM8 (F2531 Primer) | 1.25 uL |
| iGEM9 (R2531 Primer) | 1.25 uL |
| Template DNA (119.2 ng/uL) | 1 uL |
| Master Mix | 12.5 uL |
| DI Water | 9 uL |

| Component | 25 uL rxn |
|---|---|
| iGEM12 (F1224 Primer) | 1.25 uL |
| iGEM13 (R1224 Primer) | 1.25 uL |
| Template DNA | 1 uL |
| Master Mix | 12.5 uL |
| DI Water | 9 uL |

| Component | 25 uL rxn |
|---|---|
| iGEM22 (F3517 Primer) | 1.25 uL |
| iGEM23 (R3517 Primer) | 1.25 uL |
| Template DNA | 1 uL |
| Master Mix | 12.5 uL |
| DI Water | 9 uL |

<-- Need to add 1uL or less of GFP Block Template, but adding 1 uL at current concentration will yield 0.1 ng of GFP Block which seems too low??

Thermocycler:

Temp Gradient: 57-63 degrees C

Primers Tm: [59,61] degrees C